American Journal of Hematology 53:151–157 (1996)

Effects of Fibrin and α_2-Antiplasmin on Plasminogen Activation by Staphylokinase

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Staphylokinase obtains plasminogen activating activity by forming a complex with plasminogen. Although the enzymatic activity of staphylokinase is enhanced by fibrin, how fibrin enhances enzymatic activity has not been determined yet. The effects of fibrin, or fibrinogen fragments, on the activation of plasminogen by staphylokinase was investigated using CNBr-digested fibrinogen fragments (FCB-2 and FCB-5) and plasmin-degraded cross-linked fibrin fragments ((DD)E complex, DD fragments and E fragments). Kinetic analysis of the activity of staphylokinase revealed that its plasminogen activating activity, which was expressed as kcat/Km, was enhanced by FCB-2 (10-fold) and FCB-5 (5-fold). These fibrin fragments caused 38-, 30-, and 8.5-fold increases in activity for the DD fragment, (DD)E complex and E fragment, respectively. Although α_2-antiplasmin inhibited the activation of plasminogen by staphylokinase, FCB-2 abolished its inhibitory effects, and the plasminogen activating activity of staphylokinase was restored. The inhibitory effects of α_2-antiplasmin on the activation of mini-plasminogen by staphylokinase were less than for Glu- or Lys-plasminogen, and the inhibitory effect of α_2-antiplasmin was not altered by fibrin or EACA. These findings indicate that the staphylokinase/plasmin(ogen) complex reacts with fibrin even in the presence of α_2-antiplasmin, and efficient plasminogen activation takes place on the surface of fibrin. © 1996 Wiley-Liss, Inc.

Key words: staphylokinase, plasminogen activation, fibrin, α_2-antiplasmin, plasminogen

INTRODUCTION

Plasminogen activator (PA), which is a serine enzyme, converts the inactive zymogen plasminogen to the active enzyme plasmin. The digestion of fibrin by plasmin is the primary event in the fibrinolytic system [1]. PA produced in the human body can be immunologically categorized into two types: urokinase-type PA (u-PA) and tissue-type PA (t-PA). Both u-PA and t-PA cleave the Arg561-Val562 bond of the plasminogen molecule to form plasmin [2]. However, streptokinase and staphylokinase do not directly convert plasminogen to plasmin by themselves, but form a stoichiometric complex with plasminogen, and then these complexes have plasminogen activating activity [3,4].

Staphylokinase is a protein produced by *Staphylococcus aureus* [5], and the mechanism of plasminogen activation by staphylokinase has recently been investigated. Activation of plasminogen by staphylokinase proceeds through two steps [6], as it does with streptokinase. The first step is the formation of a complex between staphylokinase and plasminogen. The second is the activation of plasminogen by this complex. The complex between streptokinase and plasminogen exposes the active site of the plasminogen molecule without any proteolytic cleavage [7]. On the other hand, the PA activity of the complex between staphylokinase and plasminogen is required for the conversion of plasminogen to plasmin [6]. Kinetic studies have mainly focused on the mechanisms by which the staphylokinase/plasmin(ogen) complex activates plasminogen [8–10]. This activation is inhibited by α_2-antiplasmin [11–13], and the enzymatic activity of this complex is enhanced by fibrin [9]. The thrombolytic properties of staphylokinase in vitro are relatively more efficient than streptokinase in human plasma [4,14–16].

Received for publication May 25, 1995; accepted June 18, 1996.

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The thrombolytic efficacy of staphylokinase in animal models was greater than that of streptokinase [17,18]. Although there were immunogenic responses to intravenously administered staphylokinase in humans [19,20], staphylokinase can induce coronary thrombolysis in patients having a myocardial infarction without the concomitant induction of a systemic lytic state [21,22]. Thus, staphylokinase will be used as a thrombolytic agent in the near future, and a basic study on the effects of staphylokinase in vitro was necessary.

The mechanism of the formation of the staphylokinase/plasmin(ogen) complex on fibrin, and the activation of fibrin-bound plasminogen by staphylokinase is not clearly understood yet. In this study, the activation of plasminogen by staphylokinase on the surface of fibrin was investigated using cyanogen bromide (CNBr)-digested fibrinogen fragments (a soluble substitute for solid-phase fibrin) and plasmin degraded cross-linked fibrin fragments. Furthermore, by using proteolytically degraded plasminogen molecules, the inhibitory mechanisms of α_2-antiplasmin were examined.

MATERIALS AND METHODS

Materials

The following materials were obtained from the commercial sources indicated: H-D-Valyl-L-leucyl-L-lysine-p-nitroanilide (S-2251), streptokinase and human fibrinogen (grade L), Kabi Vitrum AB (Stockholm, Sweden); Sephadex G-100, Lysine-Sepharose and Blue-Sepharose CL-6B, Pharmacia Fine Chemicals (Uppsala, Sweden); Trasylol (aprotinin), Bayer (Leverkusen); human thrombin, human factor XIII, Greencross (Osaka, Japan); ϵ-aminocaproic acid (EACA), Seikagaku Corporation (Tokyo, Japan); Elastase, Sigma (St. Louis, MO). All other reagents and chemicals were of the highest grade available.

Purification of Proteins

Staphylokinase was produced in transformed *Escherichia coli*, and purified by ammonium sulfate precipitation and ion-exchange chromatography according to the method reported by Sako [23]. Human Glu-plasminogen was purified from fresh frozen human plasma according to the procedures described by Deutsch and Mertz [24], which utilized lysine-Sepharose affinity chromatography after ammonium sulfate precipitation, followed by gel filtration in a Sephadex G-100 gel column. The purified Glu-plasminogen contained both form I and form II. Lys-plasminogen was prepared by treating Glu-plasminogen with plasmin. Plasminogen fragments were obtained by digesting plasminogen with catalytic amounts of porcine pancreatic elastase as described previously [25]. The digested plasminogen fragments, mini-plasminogen (Val442-plasminogen), were purified by Sephadex G-75 gel filtration and lysine-Sepharose affinity chromatography. Plasminogen-free human fibrinogen was prepared using lysine-Sepharose. Human α_2-antiplasmin was purified from fresh frozen human plasma according to the procedure described by Wiman et al. [26].

The CNBr-digested fibrinogen fragment-2 and -5 (FCB-2 and FCB-5) were produced as follows [27,28]: Fibrinogen was incubated with CNBr in 70% formic acid overnight at room temperature. The FCB-2 fragments were purified by Sephadex G-100 gel filtration, and then the FCB-5 fragments were purified by Sephadex G-50 gel filtration. The purified FCB-2 and FCB-5 fragment appeared as single bands with molecular weights of 42 and 8.3 kDa, respectively. Amino acid sequence analysis of the FCB-2 fragment revealed that it is composed of five kinds of amino-terminal amino acids (fibrinogen Aα148-, Bβ191-, Bβ225-, Bβ243-, and γ95-). Amino acid sequence analysis of the FCB-5 fragment revealed that it composed of two kinds of amino-terminal amino acids (fibrinogen γ311- and γ337-).

Fibrin fragments were prepared as follows: Plasminogen-free human fibrinogen was enriched with Factor XIII and clotted as previously described [29]. The degradation products of cross-linked fibrin, the (DD)E complex, were prepared as described by Olexa and Budzynski [30]. Briefly, 100 mg of dry cross-linked fibrin and 0.1 CTA U of plasmin were suspended together in 5 ml of prewarmed 150 mM Tris-HCl buffer, containing 5 mM $CaCl_2$ and 0.02% sodium azide, pH 7.4. The digestion was performed with constant gentle stirring at 37°C for 24 hr. The reaction was then stopped by the addition of 10 μl of aprotinin to a final concentration of 10 KIU/0.1 CTA unit of plasmin, then Sepharose CL-6B gel filtration was performed. The fractions within the main peaks were combined and analyzed by SDS-PAGE and native PAGE. The main peak fraction contained pure homogeneous (DD)E complexes of 250 kDa. About 10 mg of the (DD)E complex was dissociated in 3 M urea, pH 5.3, at 37°C for 1 hr. The sample was filtered through a Sepharose CL-6B gel column. The DD and E fragments were eluted and collected separately. SDS-PAGE was performed to verify the homogeneity of the preparation. The DD and E fragment preparations, with molecular weights of 190 and 60 kDa, respectively, were pooled and concentrated.

Kinetics of Plasminogen Activation With Staphylokinase

An indirect chromogenic assay [31] was performed to obtain the kinetic parameters as follow: The activation of Glu-plasminogen or Lys-plasminogen (0.102 to 0.819 μM) by staphylokinase (16 nM) in 50 mM Tris-HCl buffer containing 0.01% Tween 80, pH 7.4, was measured at 37°C in the absence or presence of fibrinogen fragments (2 to 10 μM) or fibrin fragments (0.2 to 1.0 μM). The plasmin generated was measured with the chromogenic

substrate, S-2251 (0.6 mM). The activation obeyed Michaelis-Menten kinetics as shown by the linearity of the inverse activation rate (1/v) vs. the inverse plasminogen concentration (1/[S]) plot. The inverse of the activation rate (1/v) was plotted against the inverse of the fibrin(o-gen) fragments concentration (1/[F]). The value of 1/v at an infinite concentration of fibrin(ogen) fragments was obtained at the ordinate and plotted against the inverse of the plasminogen concentration, which yields the Michaelis constant for the activation of plasminogen by staphylokinase in the presence of fibrin(ogen) fragments.

Effects of α_2-Antiplasmin on the Activation of Plasminogen by Staphylokinase

The effects of α_2-antiplasmin on the plasminogen activation activity were investigated by SDS-PAGE under reduced conditions. Briefly, 25 μl of an equimolar mixture (20 nM) of staphylokinase with plasminogen was incubated at 37°C for 15 min with 25 μl of α_2-antiplasmin or buffer. Then this mixture was reacted with 50 μl of plasminogen (Glu-, Lys-, or mini-form) (2 μM) and incubated at 37°C for 0 to 120 min. Furthermore, the effects of FCB-2 and EACA on the inhibitory action of α_2-antiplasmin were investigated. Prior to the final reaction with 50 μl of each type of plasminogen (2 μM), 10 μl of FCB-2 (100 μg/ml) or EACA (0.4 M) was added to 25 μl of an equimolar mixture (20 nM) of staphylokinase with plasminogen, and this mixture was reacted with 25 μl of α_2-antiplasmin immediately at 37°C for 15 min. Plasminogen activation was estimated by determining the cleavage of plasmin into its heavy and light chains, as observed by SDS-PAGE under reduced conditions. The protein was visualized with Coomassie brilliant blue and the dried gel was scanned in the direction of electrophoresis with a densitometer (CS 900, Shimazu, Kyoto, Japan). Then the activation rate of plasminogen was calculated from the decreased intensity of the plasminogen band.

Analysis of the Proteins

The molecular weight of the proteins were determined by SDS-PAGE under non-reduced or reduced conditions, and native PAGE. These procedures were performed by the PHAST System (Pharmacia) using 8–25% gradient gels and native PAGE gels. The proteins were visualized with Coomassie brilliant blue. The standard molecular weight proteins used were phosphorylase b (Mr 94,000), albumin (Mr 67,000), ovalbumin (Mr 43,000), carbonic anhydrase (Mr 30,000), trypsin inhibitor (Mr 20,000), and α-lactalbumin (Mr 14,400). The amino-terminal amino acid sequences of the proteins were confirmed by protein sequencing (Model 477A, Applied Biosystems, Foster City, CA).

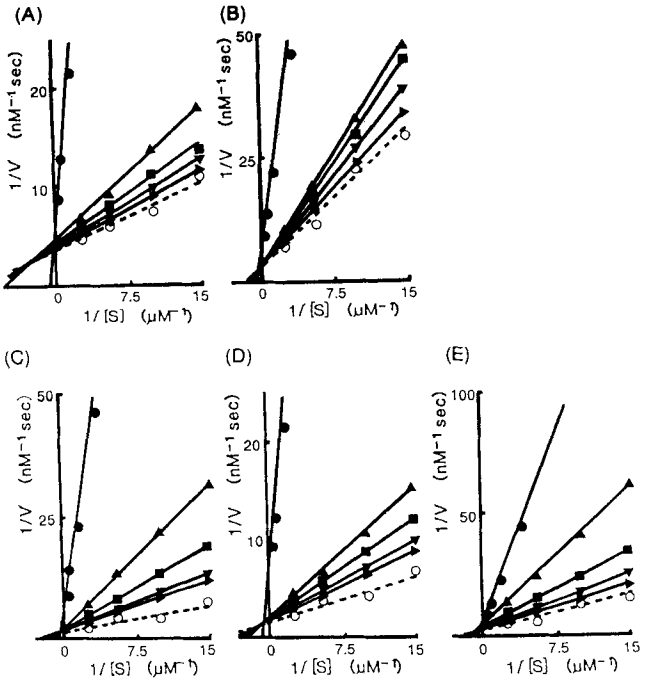

Fig. 1. Activation of Glu-plasminogen by staphylokinase in the absence and presence of CNBr-digested fibrinogen fragments and plasmin-degraded fibrin fragments. Lineweaver-Burk plots of 1/v vs. 1/[Plg] for the activation of various concentrations of plasminogen by staphylokinase in the presence of increasing concentrations of FCB-2 (A), FCB-5 (B), the (DD)E complex (C), DD fragments (D), and E fragments (E). Concentrations of FCB-2 and FCB-5 were 0 μM (●), 2 μM (▲), 5 μM (■), 7.5 μM (▼) and 10 μM (▶), respectively. Concentrations of the (DD)E complex, DD fragments, and E fragments were 0 μM (●), 0.2 μM (▲), 0.5 μM (■), 0.75 μM (▼), and 1.0 μM (▶), respectively. The values of 1/v at an infinite concentration (○) of fibrin(ogen) fragment were obtained as described in the text.

RESULTS

Kinetic Analysis of the Activation of Plasminogen by Staphylokinase in the Presence of Fibrin or Fibrinogen Fragments

Kinetic analysis revealed that Glu-plasminogen was activated by staphylokinase, and followed Michaelis-Menten kinetics, as shown by the linearity of the initial rate of activation vs. the Glu-plasminogen concentration plot (Fig. 1). The effects of CNBr-digested fibrinogen fragments on Glu-plasminogen activation by staphylokinase were investigated using FCB-2 (Fig. 1A) and FCB-5 (Fig. 1B). The kinetic parameters are summarized in Table I. Addition of FCB-2 decreased the Km value to 18% of its initial value, and the kcat value increased 1.7-fold. Addition of FCB-5 decreased the Km value by 28% and the kcat value increased 3.6-fold. Thus, the enzymatic efficiency, expressed as kcat/Km, of the activation of Glu-plasminogen by staphylokinase was increased 9.8-fold and 5.1-fold by FCB-2 and FCB-5, respectively. When

TABLE I. Kinetic Parameters for the Activation of Glu-Plasminogen by Staphylokinase*

	Km (μM)	kcat (s^{-1})	kcat/Km ($\mu M^{-1} \cdot s^{-1}$)	Ratio of kcat/Km with control
Control	1.98	0.52	0.26	1.0
FCB-2	0.35	0.89	2.54	9.8
FCB-5	1.43	1.89	1.33	5.1
(DD)E complex	0.40	3.15	7.87	30.3
Fragment DD	0.22	2.18	9.81	37.7
Fragment E	0.71	1.57	2.20	8.5

*Figures give the mean value (n = 3).

Lys-plasminogen was activated by staphylokinase, plasminogen was converted to plasmin in a time-dependent manner. Lys-plasminogen activation by staphylokinase was faster than Glu-plasminogen activation. The kcat/Km value was markedly higher for the activation of Lys-plasminogen (16.0 $\mu M^{-1} \cdot s^{-1}$) than for the activation of Glu-plasminogen by staphylokinase (0.26 $\mu M^{-1} \cdot s^{-1}$). The kcat/Km value for the activation of Lys-plasminogen was increased 3.5-fold by the addition of FCB-2 to the reaction mixture (56.1 $\mu M^{-1} \cdot s^{-1}$).

The effects of plasmin-degraded, cross-linked fibrin fragments on the activation of Glu-plasminogen by staphylokinase was investigated using the (DD)E complex (Fig. 1C) and DD (Fig. 1D) and E fragments (Fig. 1E). The Km values decreased to 20, 11, and 36% of their initial values, and the kcat values were increased 6.1-fold, 4.2-fold, and 3.1-fold by the addition of the (DD)E complex, or the DD or E fragments, respectively. Thus, the kcat/Km value was increased 30-fold, 38-fold, and 8.5-fold by the (DD)E complex, DD fragment, and E fragment, respectively. Therefore, it is clear that staphylokinase enhances enzymatic activity by reacting with fibrin-like structure.

Effects of FCB-2 and EACA on the Inhibitory Effect of α_2-Antiplasmin on the Activation of Plasminogen by Staphylokinase

The effects of α_2-antiplasmin on the activation of Glu-, Lys-, or mini-plasminogen by staphylokinase were estimated by determining the cleavage of plasmin under the reduced conditions of SDS-PAGE. Although both Glu-plasminogen form I and form II were used in this experiment, their bands overlapped and appeared as only one band on the gel (Fig. 2). Glu-plasminogen was converted, by the staphylokinase/Glu-plasmin(ogen) complex, to plasmin in a time-dependent manner (Fig. 2A); plasmin was first observed after 15 min; thereafter plasminogen activation progressed with time. All the Glu-plasminogen had been completely converted into plasmin after 120 min (Fig. 2A, lane 8). α_2-antiplasmin inhibited the activation of plasminogen by the staphylokinase/plasmin(ogen) complex (20 nM) in a dose-dependent manner (Fig. 3). α_2-antiplasmin at a concentration of 50 nM almost completely inhibited the production of plasmin (Fig. 2B).

When α_2-antiplasmin with FCB-2 (Fig. 2C) or EACA (data not shown) was added to the reaction mixture containing Glu-plasminogen and staphylokinase, the inhibitory effect of α_2-antiplasmin was abolished, and about 95.0% of the plasmin forming ability was restored (Table II). α_2-antiplasmin equally inhibited the activation of Lys-plasminogen and Glu-plasminogen by staphylokinase. EACA and FCB-2 competed with the inhibitory action of α_2-antiplasmin for Glu-plasminogen and Lys-plasminogen, comparably (data not shown). Mini-plasminogen was also activated by staphylokinase in a time-dependent manner (Fig. 2D). High doses of α_2-antiplasmin inhibited the activation of mini-plasminogen by staphylokinase (Fig. 3). However, the inhibitory effect of α_2-antiplasmin was not abolished in the presence of FCB-2 or EACA (Table II).

DISCUSSION

Staphylokinase has a plasminogen activating ability when forming the complex with plasminogen [4]. Its mode of action is similar to that of streptokinase [3], but the enzymatic specificity of the staphylokinase/plasmin(ogen) complex is different from that of the streptokinase/plasmin(ogen) complex [6]. The activation of plasminogen by the staphylokinase/plasmin(ogen) complex is inhibited by α_2-antiplasmin [11–13], and the enzymatic activity of the staphylokinase/plasmin(ogen) complex is enhanced by fibrin [9]. The mechanisms behind the fibrin-induced activation of plasminogen by the staphylokinase/plasmin(ogen) complex, and the effects of α_2-antiplasmin are not clear. In this study, we have evaluated the molecular interactions between the staphylokinase/plasmin(ogen) complex, α_2-antiplasmin and fibrin.

To analyze the effects of fibrin on the activation of plasminogen by staphylokinase, CNBr-digested fibrinogen fragments (FCB-2 and FCB-5) and plasmin-degraded cross-linked fibrin fragments, the (DD) E complex, DD and E fragments were used. The enzymatic efficiency (kcat/Km) of staphylokinase was increased about 10-fold and 5-fold by FCB-2 and FCB-5 (Table I). FCB-2 and FCB-5 are soluble substitutes for solid-phase fibrin and can accelerate t-PA mediated activation of plasminogen. Monoclonal antibodies against a part of FCB-2 (Aα-(148-160)] [32] or FCB-5 (γ-(312-325)] [33] react with fibrin, but not with fibrinogen. Moreover, Aα-Lys157 of FCB-2 and γ-Lys321 of FCB-5 bind to the lysine binding sites (LBS) of plasminogen and t-PA [27,28]. These results suggest that the enhancing effects of FCB-2 and FCB-5 are induced by the activation of their lysine-bound plasminogen as well as by the lysine-bound staphylokinase/plasmin(ogen) complex.

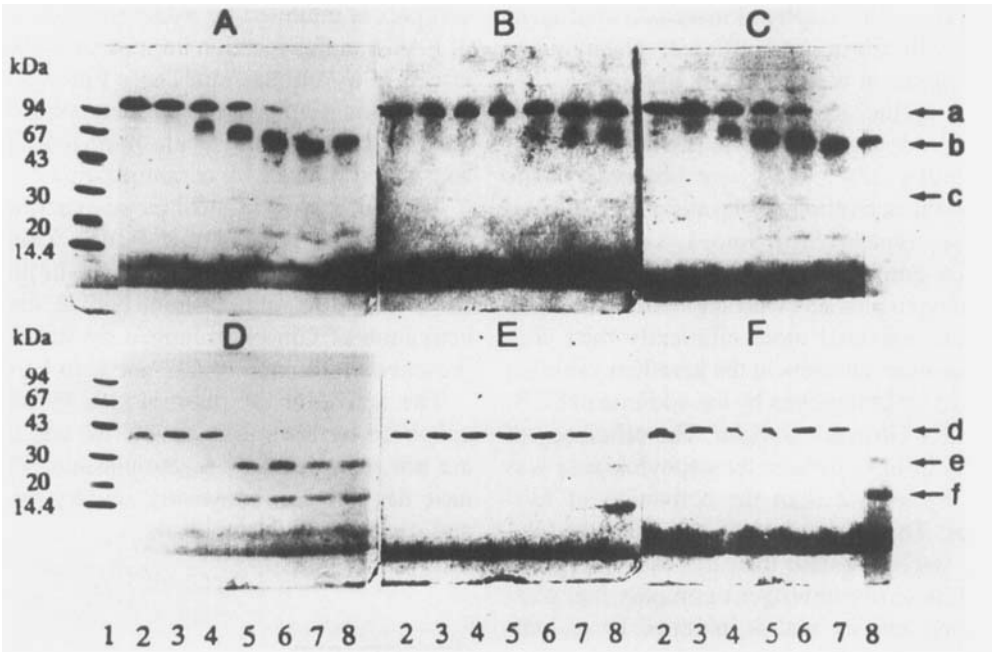

Fig. 2. Effects of α_2-antiplasmin on the activation of Glu- and mini-plasminogen by staphylokinase. The top panels show the activation of Glu-plasminogen in the absence (A) or presence (B) of α_2-antiplasmin, or in the presence of α_2-antiplasmin and EACA (C) with the staphylokinase/Glu-plasmin(ogen) complex. The bottom panels show the activation of mini-plasminogen in the absence (D) or presence (E) of α_2-antiplasmin, or in the presence of α_2-antiplasmin and EACA (F) with the staphylokinase/mini-plasmin(ogen) complex. Lanes 2, 0 min, 3, 5 min, 4, 15 min, 5, 30 min, 6, 60 min, 7, 90 min, 8, 120 min. Lane 1 contains the standard molecular weight proteins. Arrow a, Glu-plasminogen: arrow b, the heavy chain of plasmin; arrow c, the light chain of plasmin; arrow d, mini-plasminogen; arrow e, the heavy chain of mini-plasmin: arrow f, the light chain of mini-plasmin.

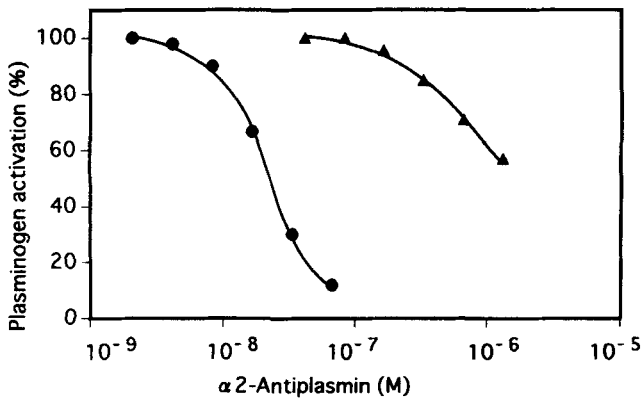

Fig. 3. The effects of α_2-antiplasmin on the activation of Glu- and mini-plasminogen by staphylokinase. Plasminogen activation was estimated by the cleavage of plasmin into its heavy and light chains observed by SDS-PAGE under reducing conditions. The degree of activation of Glu- and mini-plasminogen by staphylokinase in the absence of α_2-antiplasmin was defined as 100%, and the relative values were expressed as percentages. The mean value was obtained from 3 separate experiments. (●): Glu-plasminogen; (▲): mini-plasminogen.

TABLE II. Effects of α_2-Antiplasmin on the Activation of Glu-Plasminogen and Mini-Plasminogen by Staphylokinase*

	Control	α_2-AP	α_2-AP + FCB-2	α_2-AP + EACA
Glu-plasminogen	100	12.0	100	95.0
Mini-plasminogen	100	42.5	50.3	53.5

*Plasminogen activation was estimated by the cleavage of plasmin into its heavy and light chains, observed by SDS-PAGE under reducing conditions. The degree of activation of Glu- and mini-plasminogen by staphylokinase was defined as 100%, and the relative values were expressed as percentages. The mean value was obtained from 3 separate experiments.

Further, the enzymatic efficiency of the activation of Glu-plasminogen by staphylokinase was increased 30-fold, 38-fold, and 8.5-fold by the (DD)E complex, DD and E fragments, respectively. The plasmin-degraded cross-linked fibrin fragments contain the exposed COOH-terminal lysine residue of fibrin. When plasminogen is activated by t-PA, both proteins bind to the COOH-terminal lysine of the fibrin molecule and the plasminogen activating activity is enhanced [34]. These results suggested that the staphylokinase/plasmin(ogen) complex is bound to the lysine residue of fibrin via the LBS of plasminogen in the complex, and thus the fibrin-bound plasminogen is activated efficiently. The plasmin-degraded cross-linked fibrin fragments DD and E contain FCB-2 and FCB-

5, respectively. Thus, the staphylokinase/plasmin(ogen) complex reacts with fibrin, and efficient plasminogen activation takes place on the surface of fibrin.

The efficiency of the activation of Lys-plasminogen by staphylokinase was higher than the activation of Glu-plasminogen. Similar differences were observed in the kinetic studies on the activation of plasminogen by urokinase [35] and tissue-type PA [36]. Since Lys-plasminogen has a more open conformation than Glu-plasminogen [37], Lys-plasminogen interacts with staphylokinase more easily, and is thus activated more efficiently than Glu-plasminogen. However, changes in the kcat/Km value for the activation of Lys-plasminogen by the addition of FCB-2 were less than for Glu-plasminogen. The efficiency of the activation of Glu-plasminogen by staphylokinase was enhanced more by FCB-2 than the activation of Lys-plasminogen was. This is due to less activation of Glu-plasminogen by staphylokinase than Lys-plasminogen.

The staphylokinase/plasmin(ogen) complex has plasminogen activating activity that is inhibited by α_2-antiplasmin. In this case, the α_2-antiplasmin forms a complex with the staphylokinase/plasmin(ogen) complex via the LBS as well as the active site of the plasmin molecule in the complex. When the enzymatic activity of staphylokinase in the plasma was measured by the digestion of fibrinogen, and plasmin/α_2-antiplasmin complex production, minimal fibrinogen digestion and no plasmin/α_2-antiplasmin complex production was observed in the presence of fibrin [14]. The inhibition of the staphylokinase/plasmin(ogen) complex by α_2-antiplasmin relies on the availability of the LBS of plasminogen in the complex. This is supported by the finding that α_2-antiplasmin inhibition of the staphylokinase/mini-plasmin(ogen) complex, which lacks the high affinity LBS (in kringle 1 - kringle 4) of plasminogen, is about 100-fold lower than the inhibition of the staphylokinase/Glu-plasmin(ogen) complex (Fig. 3). Furthermore, addition of EACA (a lysine derivative) or FCB-2 did not influence α_2-antiplasmin inhibition of the staphylokinase/mini-plasmin(ogen) complex. Since the LBS is found in Glu-plasminogen and not in mini-plasminogen, binding of α_2-antiplasmin to the LBS is vital to the inhibitory effect of α_2-antiplasmin. The critical role of the LBS was also stated in a previous report [26]. Therefore, EACA or FCB-2 that binds to the LBS interferes with the reaction between α_2-antiplasmin and plasmin, and thus suppresses the action of α_2-antiplasmin.

The mode of action of CNBr-digested fibrinogen fragments, or EACA, was kinetically analyzed by Lijnen et al. [11], and they reported that addition of CNBr-digested fibrinogen fragments and EACA to the reaction mixture of Glu-plasminogen and α_2-antiplasmin resulted in a concentration-dependent reduction of the apparent inhibition rate constant. As shown in Figure 2, the plasminogen activating activity of the staphylokinase/plasmin(ogen) complex is inhibited by α_2-antiplasmin, and the presence of EACA in this reaction mixture abolished the inhibitory effects of α_2-antiplasmin. These findings indicate that the staphylokinase/plasmin(ogen) complex binds to fibrin, and that this complex has plasminogen activating activity, and is not affected by α_2-antiplasmin.

The buffer used in this kinetic experiment was 50 mM Tris-HCl buffer containing 0.01% Tween 80, pH 7.4. Since Cl^- inhibits the activation of Glu-plasminogen [38], the effects of α_2-antiplasmin, FCB-2, and EACA on the activation of Glu-plasminogen by staphylokinase in the presence or absence of Cl^- needs to be studied further.

The activation of plasminogen by staphylokinase is enhanced by fibrin-like structures, and these complexes are not suppressed by α_2-antiplasmin. Thus, staphylokinase has efficient enzymatic activity on fibrin surfaces, and can induce thrombolysis.

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